ammonium di hydrogen orthophosphate, and potassium nitrate.

10. (Amended) The method of claim 3 wherein the sterilized medium further comprises an organic nutrient selected from the group consisting of malt extract, yeast extract, potato extract, peptone, soya-peptone, bactopeptone, and corn steep liquor.

a4 cont.

11. (Amended) The method of claim 3 wherein the sterilized medium further comprises a detergent selected from the group consisting of Tween-20, Tween-80, and Tween-100.

12. (Amended) The method of claim 3 wherein the enzyme preparation containing high cellobiase activity also contains high endo-glucanase activity and high cellobiohydrolase activity.

Remarks

This paper amends the title, specification and claims, canceling Claims 1, 2, 4, and 5 and amending claims 3 and 6-12. Claims 3 and 6-12 remain pending in the case.

The amendment of the title reflects the focus of the claims as amended on the method of enhancing cellobiase activity. The amendment of the specification and Claim 3 to delete reference to the specific strain of *Termitomyces clypeatus* and the ATCC deposit, reflects the focusing of the claims on the methods of producing an enzyme preparation which are applicable to *Termitomyces clypeatus* strains in general. Support can be found at least on page 4, lines 10-13 and page 5, lines 2-5 in the specification as filed.

In Claim 3, reference to a method for producing an enzyme preparation containing high cellobiase activity is supported at least by Claim 3 as originally filed; on page 1, lines 8-10; and on page 4, lines 10-13.

Reconsideration and withdrawal is requested of the rejection of Claims 1-12 under 35 U.S.C. 112. Cancellation of Claims 1, 2, 4 and 5 obviates the rejection of those claims. In the remaining Claims 3 and 6-12, reference to a specific strain of *Termitomyces clypeatus*, which has been deposited, has been deleted. The invention in Claims 3 and 6-12 is, thus, directed to a method of producing an enzyme preparation containing high cellobiase activity using a glycosylation inhibitor without reference to a specific deposit strain. Thus, it is the presence of the glycosylation inhibitor in the medium that is the basis of the invention as now claimed rather than the specific strain of *Termitomyces clypeatus*.

The focus of the invention as now claimed, on the method rather than the microorganism is brought out on page 8, paragraph 3, line 1 of the application. Further, Tables 1 & 2 show that the cellobiase activity was enhanced upon addition of 2-deoxy-D-glucose. For instance, in Table 1, the control medium with 2-deoxy-D-glucose at 0.05 mg/ml, produced 2.236 units of cellobiase activity per ml, whereas the control medium with 2-deoxy-D-glucose at 1 mg/ml produced 50.097 units cellobiase activity per ml. Similarly, Table 2 shows that the control medium with 2-deoxy-D-glucose gave 50.097 units cellobiase activity per ml and control medium with 2-deoxy-D-glucose and mannose resulted in increase in cellobiase activity to 140.60 units/ml. Because the invention is not limit by recitation of a specific strain of *Termitomyces clypeatus*, which has been deposited, no microorganism deposit or reference to such deposit in the claims or specification is believed to be required.

Reconsideration and withdrawal of the rejection of Claims 1-12 under 35 U.S.C. 112, second paragraph is requested in view of the cancellation of Claims 1, 2, 4 and 5 and the amendments to Claims 3 and 6-12, most of which were suggested by the P.T.O.

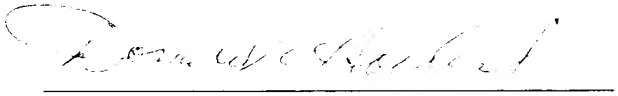
In particular, in Claim 3 the term "(the edible mushroom)" has been deleted. In Claim 6, the words "the strain is cultivated on" have been deleted, "containing" has been changed to "contains" and the words "in the presence of glycosylation inhibitors" have been deleted. Claim 7 has been amended to recite that "the assimilable carbon sources

used are carbohydrates , agrowastes, TCA cycle acids, amino acids, or glucose analogue D-glucosamine' and of each of these are further described in the claim where appropriate. Also, Claim 7 is amended to depend from Claim 6 as suggested. In Claim 8, reference to glycosylation inhibitors in absence of such recitation in independent Claim 3, has been corrected by amending Claim 3 to reference glycosylation inhibitors. In Claim 9, the dependency is changed to Claim 6 in which "assimilable carbon and nitrogen sources" is recited. In Claim 10, the dependency is changed from Claim 13 to Claim 3. Claim 11 is amended to provide that the sterilized medium further comprises a detergent so that reference to detergent is not required in Claim 3. Claim 12 is amended to delete reference to the fungi name and to recite that "the enzyme preparation containing high cellobiase activity also contains high endo-glucanase activity and high cellobiohydrolase activity".

Reconsideration and withdrawal of the rejection of claims 1 and 2 under 35 U.S.C. 102(b) is requested in view of the cancellation of said claims by this paper.

It is believed that the amendments herein place the claims in a condition for allowance and such favorable response is requested. If, however, any of the claims are considered by the P.T.O. to be not in a condition for allowance, the P.T.O. is requested to contact the undersigned attorney to resolve any remaining issues.

Respectfully submitted,



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SPECIFICATION AMENDMENTS IN MARKED UP VERSION:

Please delete the title on page 1, lines 1-2 and substitute therefor, replacement title as follows.

A method for enhancing Cellobiase activity of [the novel strain] Termitomyces clypeatus using [2-deoxy-D-glucose as] a glycosylation inhibitor.

Please delete the paragraph starting on page 5, line 14 and substitute therefor, the replacement paragraph as follows.

Accordingly, the present invention provides a method for enhancing the cellobiase activity of the strain *Termitomyces clypeatus* using 2-deoxy-D-glucose as glycosylation inhibitor which comprises [of] inoculating and growing mycelial culture of [(] the edible mushroom [)] , *Termitomyces clypeatus*, (having the accession number IICB-411, given by Indian Institute of Chemical Biology, Calcutta, India, a constituent laboratory of the applicants [and being deposited at ATCC and having accession No.....]), in sterilized medium containing 2-deoxy-D-glucose, cellobiose, ammonium di hydrogen phosphate and conventional micronutrients at pH between 3-8 and incubating at temperatures between 20-35°C under shaking in aerobic conditions and separating the culture filtrate by known methods, using the culture filtrate directly as the source of enzyme cellobiase, endo-glucanase and cellobiohydrolase for use in cellulose hydrolysis.

Please delete the paragraph starting on page 6, line 18 and substitute therefor, the replacement paragraph as follows.

In another embodiment of the present invention, the mycelial culture of the edible mushroom *Termitomyces clypeatus* having accession number IICB-411, given by Indian Institute of Chemical Biology, Calcutta, a constituent laboratory of Council of Scientific and Industrial Research, India [and also being deposited at ATCC and having accession No. .....] is used.

CLAIM AMENDMENTS IN MARKED UP VERSION:

Please cancel claims 1, 2, 4 and 5 without prejudice or disclaimer.

Please amend claims 3-5 and 6-12 as follows.

3. (Amended) A method for producing an enzyme preparation containing high cellobiase activity [enhancing the cellobiase activity of strain *Termitomyces clypeatus*, using 2-deoxy-D-glucose as glycosylation inhibitor] , said method comprising [steps of]:

(a) [obtaining a culture medium of (the edible mushroom) *Termitomyces clypeatus* (having an accession number IICB-411 given by Indian Institute of Chemical Biology, Calcutta, constituent laboratory of the applicants being deposited at ATCC and will be given reference no .....) by] inoculating [and growing] a mycelial culture of a strain of *Termitomyces clypeatus* [in] into sterilized medium containing at least 0.05 [to 5.0] mg/ml of a glycosylation inhibitor [2-deoxy-D-glucose] at pH between 3 to 8 ; (b) [and] incubating at temperatures between 20-37°C under shaking in aerobic conditions [, (b)] ; and (c) separating the culture medium from the mycelia to produce the enzyme preparation containing high cellobiase activity [by known methods, and (c) using the culture filtrate directly as the source of the enzyme cellobiase and also for endo-glucanase and cellobiohydrolase for use in cellulose hydrolysis].

6. (Amended) [A process as claimed in] The method of claim 3 wherein [, the strain is cultivated on] the medium [containing] contains assimilable carbon and nitrogen sources, inorganic salts and organic nutrients [, in presence of glycosylation inhibitors].

7. (Amended) [A process as claimed in] The method of claim [3] 6 wherein [,] the assimilable carbon sources used are carbohydrates , agrowastes, TCA cycle acids, amino acids, or glucose analogue D-glucosamine wherein the carbohydrates are selected from the group consisting of cellobiose, mannose, fructose, xylose, arabinose, starch, dextrin, cellulose, cotton, xylan [and/or] ; wherein the agrowastes are selected from the group

consisting of baggasse powder, rice-straw powder, wheat bran, corn cob powder, corn powder ; wherein the [, in presence of] TCA cycle acids are selected from the group consisting of succinate, fumarate, and maleate [or] ; and wherein the amino acids are selected from the group consisting of aspartate, glutamate, serine, histidine , and alanine [or glucose analogue D-glucosamine].

8. (Amended) [A process as claimed in] The method of claim 3 wherein [,] the glycosylation inhibitor [inhibitors] is selected from the group consisting of tunicamycin, dexoy nojirimycin, 2-deoxy-D-glucose and D-glucono-lactone.

9. (Amended) [A process as claimed] The method of claim [3] 6 wherein [,] the assimilable nitrogen source is [sources used are] selected from the group consisting of ammonium chloride, ammonium nitrate, ammonium di hydrogen orthophosphate, and potassium nitrate.

10. (Amended) [A process as claimed in] The method of claim [13] 3 wherein the sterilized medium further comprises an [, the] organic nutrient [nutrients used are] selected from the group consisting of malt extract, yeast extract, potato extract, peptone, soya-peptone, bactopeptone, and corn steep liquor.

11. (Amended) [A process as claimed in] The method of claim 3 wherein the sterilized medium further comprises a detergent [, detergents used are] selected from the group consisting of Tween-20, Tween-80, and Tween-100.

12. (Amended) [A process as claimed in] The method of claim 3 wherein [,] the enzyme preparation containing high cellobiase activity also contains high [the strain *Termitomyces clypeatus* also provides] endo-glucanase activity and high cellobiohydrolase activity [for hydrolysis of cellulose. to glucose].